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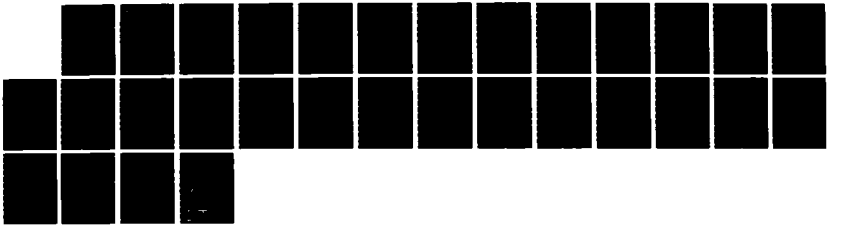

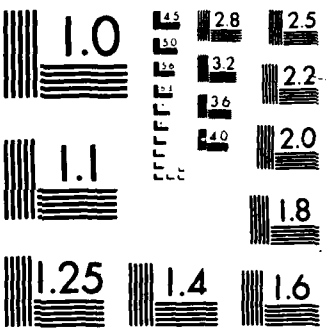
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Second International Workshop on Neuroimmunomodulation

Claire E. Zomzely-Neurath

September 18, 1986

U.S. Office of Naval Research, London

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The presentations at this workshop emphasized the close interrelationships of the nervous and immune systems. There were 10 sessions: neuroendocrine correlates of neuroimmunomodulation; enkephalins endorphins: immunomodulators; neuroendocrine receptors in the immune system; natural mediators and pharmacology of neuroimmunomodulation; neurophysiological correlates of neuroimmunomodulation; stress and immunity; sleep and immune functions; behavior, associative learning, and memory; cancer, AIDS, and immunity; psychiatric and neurological disorders with immunological features.

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SECOND INTERNATIONAL WORKSHOP ON NEUROIMMUNOMODULATION

1 INTRODUCTION

The Second International Workshop on Neuroimmunomodulation was held at the Hotel Libertas in Dubrovnik, Yugoslavia, from 1 through 6 June. B.D. Jankovic (Yugoslavia) was chairperson of the organizing committee; he was assisted by N.A. Spector (US) and W. Pierapaoli (Switzerland and Italy). About 150 scientists, including speakers, attended this focused and intensive workshop-conference on a rapidly expanding area of research on the interrelationship between the nervous and immune systems. The participants were from 23 countries, attesting to the worldwide interest in the subject of neuroimmunomodulation. American scientists had the largest representation, 25 percent of the total participants.

The scientific program consisted of 10 sessions with invited speakers as well as short oral presentations. In addition, there were 48 posters related to the session topics. The scientific sessions covered a wide range of topics including neuroendocrine effects, neuroendocrine receptors, pharmacological and neurophysiological aspects, stress, and behavior as well as psychiatric and neurological disorders with immunological features. The detailed format of the scientific program is shown in Table 1 (see page 21).

A summary of selected topics from the workshop-conference on neuroimmunomodulation is presented in the following section. It is not possible to cover all the lectures in this report. However, the proceedings of this conference will be published in full by the New York Academy of Sciences and is expected to be available in early 1987.

2 NEUROENDOCRINE CORRELATES OF NEUROIMMUNOMODULATION

Brown Adipose Tissue and the Immune System

B.D. Jankovic (Immunology Research Center, Belgrade, Yugoslavia) presented studies on the *in vivo* interrelationships between brown adipose tissue and the immune system. Brown fat in contrast to the well-known white fat contains a rich network of blood capillaries and more glycogen as well as less lipid than white fat. Brown fat is very important in hibernating animals, whereas in nonhibernating animals (for example, rat) it is present in the newborn animal but disappears during development to the adult. Thus the brown fat is involved in thermoregulation.

Jankovic and coworkers carried out extensive experiments in rats which had been adipectomized (surgical excision of the interscapular brown adipose tissue at birth), thymectomized (neonatal removal of the thymus) as well as adipectomized, and thymectomized and corresponding sham-operated controls. The following immunological models were employed:

- Antibody production to soluble and corpuscular antigens
- Arthus and delayed hypersensitivity skin reactions to bovine serum albumin
- Rejection of allogeneic skin and thyroid grafts
- Lymph node enlargement array
- Experimental allergic encephalomyelitis and thyroiditis
- Treatment of normal animals with extracts from brown adipose tissue
- Allergic encephalomyelitis in thymo-adipectomized rats
- Influence of met-enkephalin and leu-enkephalin on humoral immune responses.

The survival rate of adipectomized mice innoculated with Sarcoma cells was also evaluated. The following results were obtained:

1. Cell-mediated immune reactions were potentiated in adipectomized rats, but not antibody production.
2. Adipectomized mice innoculated with Sal tumor cells survived longer than controls; i.e., adipectomy made possible the recognition of even discrete histocompatible differences between Sal cells and A/JAX mice.
3. Adipectomy increased the ability of rats to develop autoimmune diseases.

4. Saline extracts from brown adipose tissue of newborn rats suppressed hypersensitivity skin reactions in normal adult rats.

5. Thymo-adipectomized rats showed an almost normal ability to develop allergic encephalomyelitis, a finding which indicated that the potentiating influence of adipectomy on encephalomyelitis was neutralized by thymectomy and vice versa.

6. It appears that the brown adipose tissue is a natural antogonist of the thymus.

7. Enkephalins were found to be more effective immunosuppressors in adipectomized animals than in normal ones.

The last finding establishes a link between brown adipose tissue and neuropeptides. These results indicated that the brown adipose tissue may be associated into an integrated immuno-neuroendocrine system.

Lymphocyte Environment-Sensing Mechanisms

J.W. Hadden (Immunopharmacology Program, University of South Florida Medical College, Tampa, Florida) discussed the mechanisms by which lymphocytes "sense" their environment. Lymphocytes are exposed *in vivo* to a variety of environmental and hormonal influences including neurotransmitters, hormones, and inflammatory mediators which modulate their functions in response to immunologically specific antigenic stimuli.

It appears that one way or another, the mechanisms of hormone action--i.e., the cyclic nucleotides, membrane ATPases, nuclear processes, and calcium influx--are involved in the expression of many of the specific and nonspecific stimuli. As a general rule, lymphocyte functions appear to be modulated in a positive way by cyclic GMP related mechanisms and in a positive way by cyclic AMP. Recent studies into the mechanisms of activation of these cells point to a central role for release and conversion of arachidonic acid. The conversion of arachidonic acid to prostaglandins via the cyclo-oxygenase pathway acts in negative feedback mechanisms via cyclic AMP. Conversion to hydroxy and hydroperoxy eicosatetraenoic acids, via the lipoxygenose pathway, acts in positive activating mechanisms via cyclic GMP. Activators of the cyclic AMP system in lymphocytes include prostaglandins, β-adrenergic agents, thymic hormones, and endotoxins. Activators of the cyclic GMP system include eicosanoids, cholinergic agents, thymic hormones, interleukin-2, and endotoxins. Calcium influx and mobilization appear to be central to lymphocyte activation. Hormones such as insulin, growth hormone, and thyroid hormone are more complicated in their actions and appear to provide the background which allows and supports the actions of immunologic stimuli and specific hormones of the system. Recent evidence indicates that the regulation of T-cell ontogeny involves a series of actions of thymic epithelial cell products (including thymic hormones) and of interleukins. Indirect evidence implicates central nervous system (CNS) links in this scheme. Hadden believes that CNS-directed hormonal and neurogenic influences are perhaps far more critical than suspected in the maintenance and regulation of *in vivo* immune responses.

The Thymus as a Neuroendocrine Organ

V. Geenen, J.J. Legros and P. Franchimont (Neuroendocrine Unit, Université de Liège, Belgium) spoke about their work on the thymus as a neuroendocrine organ. Recent studies have identified distinct cell populations of the thymus through the use of a monoclonal antibody (A2B5) which usually recognizes a complex ganglioside expressed on the membrane of neurons, neural crest-derived, and neuropeptide-secreting cells. However, the presence of neurohormones in the thymus had not been systematically investigated. The recent studies of Geenen et al. tackled this question.

Immunoreactive oxytocin (OT) and neurophysin (Nph) were detected and quantified by specific radioimmunoassays in human thymus extracts. Serial dilutions of extracts paralleled the appropriate standard curves. Thymus-extracted OT and Nph eluted in the same positions as a reference preparation on Sephadex G-75. Authenticity of OT was further confirmed

using biological assay and high-pressure liquid chromotography. In most cases, thymus contents of OT and Nph were greater than expected from known circulating levels and declined wtih increasing age. The molar ratio of OT/Nph in thymus was similar to that found in the hypothalamo-neurohypophyseal system, which strongly suggested a local synthesis of OT. Immunohistochemical studies revealed Nph-containing cells in the thymic epithelium of the medulla and the subscapular cortex. In a myasthenic young male, thymic OT content and molar ratio of OT/Nph were far greater than in normal patients. Geenen et al. concluded that these findings provide the demonstration of a neuroendocrine function integrated in the human thymus and suggest a role for OT and related neuropeptides in thymocyte differentiation or proliferation. OT and vasopressin were shown to replace interleukin-2 (the T-cell growth factor for γ-interferon production by mouse splenocytes). Thus, several neuropeptides may play a role in the control of cell proliferation. Geenen et al. hypothesize that some comitogenic, inductive, or repressive actions of OT may be involved in thymic lymphocyte differentiation or proliferation.

The Pineal Gland and Immunity

G.J.M. Maestroni, A. Conti, and W. Pierpaoli (Instituto Cantonale di Patologia, Locarno, Switzerland and Institute for Integrative Biomedical Research, Ebmatingen, Switzerland) reported on the role of the pineal gland in immunity. Scant studies exist on the role of the pineal gland in immunity and most of those that do report experiments performed in surgically pinealectomized mice. Maestroni et al. considered the surgical approach to be inadequate in such studies and therefore used a pharmacologic approach.

In this approach, Maestroni et al. inhibited the night surge of pineal melatonin by evening administration of the beta-blocker, propanolol, or by daily injections of para-chlorophenylanine in mice. Humoral and cell-mediated immune reactions were then tested in these mice and compared with responses of normal animals. Both *in vivo* antibody production against T-dependent antigens and the autologous mixed lymphocyte reaction were found to be significantly depressed in the pharmacologically pinealectomized mice. This depression was completely reversed by exogenous melatonin. Furthermore, when injected in normal mice, melatonin enhanced primary antibody production and was able to counteract the suppressive effect induced by corticosterone. However, melatonin failed to affect immune responses when added directly *in vivo*, and no specific binding of H^3-melatonin could be found in mouse and human lympho-hemopoietic cells. This suggested to Maestroni et al. that its augmenting action is possibly mediated by other neuroendocrine mechanisms. In fact, naltrexone completely abrogated the immunostimulating effect of melatonin, indicating that opiates are involved in its action. All together these results point to a primary immunomodulatory role of the pineal gland. Maestroni et al. consider that these findings offer the basis for developing new, interesting, and physiological immunotherapeutic interventions.

Neuroendocrine Cells in Immune Tissue

R. Hogue-Angeletti and W.F. Hickey (University of Pennsylvania Medical School, Philadelphia) presented preliminary data on neuroendocrine cells in immune tissue. They have identified a neuroendocrine cell type present in low numbers in the spleen, lymph node, and fetal liver of the rat. The cells from spleen were purified to give a uniform population of cells. There are approximately 100 to 200,000 cells per rat spleen. This very low number is probably the reason that these cells had not been detected previously. The cells are large, with abundant cytoplasm and possess large, dense-cored vesicles as seen by electron microscopy. When incubated with neutral red, the cells rapidly take up the dye as do chromaffin (neuroendocrine) cells. Preliminary data indicate that these purified spleen cells can survive in short-term tissue culture. A battery of immunological markers have been used to define

the cell type within the context of the immune system. The secretory products of those cells are being examined for potentially biologically active polypeptides. Hogue-Angeletti and Hickey postulate that the cells may be important in immune regulatory function and may form an added link between the nervous and immune systems.

Immunoregulatory Feedback

A. del Rey, H. Besedovsky, E. Sorkin, and C. Dinarello (Swiss Institute for Medical Research, Davos, Switzerland, and Tufts University School of Medicine, Boston, Massachusetts) presented their studies concerned with an immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. The production and action of immunoregulatory cytokines, including interleukin-1 (IL-1), are known to be inhibited by glucocorticoid hormones *in vivo* and *in vitro*.

A. del Rey et al. reported on a reciprococal situation in which IL-1 stimulates ACTH and glucocorticoid release *in vivo*. Antibody to IL-1 neutralizes the glucocortoid, increasing activity of factors released by virus-stimulated human leukocytes. Administration of subpyrogenic doses of homogenous human monocyte-derived IL-1 or of the pI 7 form of human recombinant IL-1 to mice and rats were found to increase ACTH and corticosterone blood levels. These results strongly suggest that an IL-1 mediated glucocorticoid increase constitutes part of a normal host response to environmental antigenic stimuli, according to del Rey et al. This endocrine response reflects the existence of an immunoregulatory feedback circuit involving the monokine IL-1 and the glucocorticoid increasing factor (GIF) of lymphoid origin as afferent and glucocorticoid as efferent hormonal signals. According to these researchers, such glucocorticoid-associated immunoregulatory mechanisms may exert a continuous surveillance of immunological cell mass and activity, and thereby prevent an excessive cummulative expansion of immunological cells that could then favor autoimmune and lymphoproliferative diseases. A. del Rey et al. think that overstimulation of the pitutary-adrenal axis may, however, contribute to pathological states. For example, a massive and sustained release of IL-1, causing increased glucocorticoid blood levels may be one of the factors mediating the immunosuppression observed during the actute phase of several infectious diseases, thus favoring superinfection.

Identification of Pro-Opiomelanocortin mRNA

H.J. Westly, A.J. Kleiss, K.W. Kelly, P.K.Y. Wong, and P.H. Yuen (Department of Animal Science and Medical Microbiology, University of Illinois, Urbana) presented their recent work on the identification of pro-opiomelanocortin messenger RNA (mRNA) in Newcastle Disease Virus-infected murine splenocytes.

Pro-opiomelanocortin (POMC) is the precursor protein for adrenocorticotropin (ACTH), α-β-γ-melanotropin, β-endorphin, and β- and γ-lipotropin. Recent evidence suggests that lymphocytes that are infected with viral or bacterial agents sythesize immunoreactive ACTH and β-endorphin coordinately with γ-interferon. Westly et al., using indirect immunofluorescent techniques with an antibody to ACTH (1-39), have confirmed these earlier results. They have now shown that virus-infected splenocytes express mRNA that codes for the synthesis of POMC and that this large precursor molecule is probably the source of splenocyte-derived ACTH and β-endorphin. Total cellular and poly (A+) cytoplasmic RNA were isolated from a murine ACTH-secreting pituitary cell line (AtT-20) and from murine splenocytes that were incubated for 18 hours in the absence or presence of Newcastle disease virus (NDV). Increasing concentrations of both total cellular and poly (A+) cytoplasmic RNA were blotted onto gene screen membranes and hybridized overnight with the recombinant plasmid, pmk Su 16 that was labeled with ^{32}PdCTP. This plasmid contains the entire POMC-coding sequence which was isolated from the mouse AtT-20/D_{16}v cell line. There was positive hybridization of the probe to total cellular and poly (A+) cytoplasmic

RNA from the AtT-20 cell line and NDV-infected splenocytes. This probe did not hybridize to either total cellular or poly (A+) cytoplasmic RNA from noninfected splenocytes or to total cellular RNA isolated from mouse liver. The ^{32}PdCTP-labeled pBR322 plasmid without the POMC insert did not hybridize with RNA from any of the cells. Northern blot analysis showed that the size of poly (A+) cytoplasmic RNA from NDV-infected splenocytes was identical to the size of mRNA from AtT-20 cells. These data provide direct evidence that splenocytes can be activated to synthesize pituitary-like hormones.

Treatment of Hormone Dependent Cancers with Analogs of Hypothalamic Hormones

Clinical studies on the treatment of hormone-dependent cancers with analogs of hypothalamic hormones were presented by A.V. Schally (VA Medical Center and Tulane University School of Medicine, New Orleans, Louisiana). This is a new approach to the treatment of endocrine-dependent or hormone-sensitive tumors based on analogs of LH-RH (leutinizing hormone releasing hormone) or somatostatin analogs. The successful use of agonistic analogs of LH-RH for the treatment of androgen-dependent prostate cancer was first demonstrated experimentally by Schally and coworkers and has now been documented in several hundred patients. Experimental studies suggest that agonists of LH-RH might be useful for treatment of estrogen-dependent breast cancer, and Schally presented evidence from clinical studies which support this view.

On the basis of experimental findings, Schally said that LH-RH agonists could also be considered as adjuncts for therapy of pituitary tumors, chondrosarcomas, osteosarcomas, and pancreatic cancer, but that the principal approach for the treatment of these neoplasms might be based on somatostatin analogs. In turn, somatostatin analogs can serve as adjuncts in treatment of prostatic cancer and breast cancer. Schally presented data showing that long-acting delivery systems for once-a-month adminstration of microcapsules of D-trp-6-LH-RH have been successfully combined with some chemotherapeutic agents such as Novatrone in the experimental treatment of prostatic cancer. Work is in progress on the use of LH-RH analogs for treatment of ovarian cancer, neoplasms of the female genital tract, myomas, endometrial carcinoma, and for protection against gonadal damage during chemotherapy and radiation. Schally thinks that methods based on the use of LH-RH agonists and somatostatin analogs might supplement, or in some cases, replace conventional procedures for the treatment of hormone-sensitive cancers.

3 ENKEPHALINS, ENDORPHINS: IMMUNOMODULATORS

Effects of Methionine Enkephalin on the Immune System

J. Wybran and L. Schandené (Department of Immunology and Hematology, Hospital Erasme, Free University of Brussels, Belgium) presented their studies on the effects of methionine-enkephalin (Met-Enk) upon the human immune system. Met-Enk is an endogenous opioid peptide acting mainly on the nervous system. *In vitro* studies of the effect of Met-Enk on the human immune system by Wybran and Schandené showed that T-lymphocytes have receptors for Met-Enk. Met-Enk was found to increase the appearance of various surface markers like T10, Tac and Leu 11. Functionally, Met-Enk significantly enhanced natural killer (NK) activity and IL-2 production. It also induces the production of NK-enchancing factors.

Based on the *in vitro* data, Wybran and Schandené carried out an *in vivo* (clinical) study on patients with AIDS. Met-Enk was administered, without any toxic side effects, for 2 to 6 weeks to six pre-AIDS and AIDS patients in order to assess the immune effects. All the patients showed a major increase in NK activity (from a mean of 16 percent before treatment to a mean of 35 percent after Met-Enk; $p<0.01$) and an increase in mitogen response. Depending upon the dose of Met-Enk used, the T_4 subset increased during treatment (e.g., from 20 to 90 percent and 12 to 21 percent). According to Wybran and Schandené the

results indicate that Met-Enk stimulates cell-mediated immunity both *in vitro* and *in vivo* and as such may be used as an immunorestorative agent. Further studies are in progress in the use of Met-Enk in the treatment of AIDS. Wybran and Schandené think that Met-Enk is likely to play a physiological role in the relations existing between the immune, endocrine, and nervous systems.

Opiate-Induced Suppression of Antibody Production

R.J. Weber, A. Pert, K.C. Rice and A.A. Hagan (NIMH and NIADDK, Bethesda, Maryland, and American University, Washington, DC) spoke on their research on opiate-induced suppression of antibody (Ab) production *in vivo*. They studied the effects of opiates on the *in vivo* Ab response to T-dependent and T-independent antigens. Weber et al. found that chronic adminstration of natural (-)-morphine induces an immunosuppression of an early event in the T-dependent primary Ab response to trinitrophenyye^{40}-ovalbumin (TNP-OVA). This opiate-mediated immunosuppression was naloxone-reversible and stereospecific, properties which suggest that the morphine-induced immunosuppression is mediated through an opiate receptor. Weber et al. think that the above results in conjunction with previous reports by others on the presence of endorphins in the central and peripheral nervous systems and in extracts of hypothalamus and adrenals make it likely that endogenous opiate may be important neuroendocrine modulators of immune response *in vivo*.

Immunomodulating Activities of Enkephalins

B.D. Jankovic and D. Maric (Immunology Research Center, Belgrade, Yugoslavia) reported on their studies designed to examine immunomodulating activities of enkephalins. Their work encompassed humoral immunity and cellular immunity on mice and rats. For the study of *in vivo* suppression of humoral immune response, BALB/c mice and Wistar rats were treated with different doses of Met-Enk and leu-enkephalin (Leu-Enk) at different time intervals before and after immunization with sheep red blood cells (SRBC). Enks were given either intraperitoneally (i.p.) or intracerebroventricularly (i.c.v.) via a cannula permanently inserted in the lateral ventricle of the brain. Four days after immunization, the number of splenic plaque-forming cells (PFC) and titers of circulating anti-SRBC antibody were determined. Jankovic and Maric found that immune responses were suppressed in all groups of mice treated with Enks. The most striking suppression (i.e., a seven-fold decrease in the number of PFC) was obtained in the experimental group in which mice were treated i.p. with 10 mg/kg body weight (b.w.) of Met-Enk and Leu-Enk 4 days before and 4 days after immunization with SRBC. Met-Enk and Leu-Enk were equally active in inducing immunosuppression in mice. A single administration of Enks at a dose of 10 mg/kg b.w. either before or after immunization did not affect the PFC response and antibody production. Blood cell counts revealed a decrease in the number of leucocytes and lymphocytes in Enk-treated mice. A similar suppression of humoral immunity was observed in rats treated i.p. with 5 mg/kg b.w. of Met-Enk or Leu-Enk. However, i.c.v. treatment with Enks, even with a lower dose of pentapeptides, was more effective in inducing immunosuppression. In rats, Met-Enk was more immunosuppressive then Leu-Enk. These results suggest that Enks are potent immunomodulators in mice and rats. Furthermore, the striking decrease of humoral immunity in rats treated i.c.v. with Enks indicates that Enks can also modulate immune response via CNS mechanisms.

In another study by Jankovic and Maric designed to examine the effects of Met-Enk and Leu-Enk on cell-mediated immune reactions, immunized Wistar rats with 0.5 mg of bovine serum albumin (BSA) in complete Freund's adjuvant. The first i.p. injection of 5 mg/kg b.w. of Met-Enk or Leu-Enk was given on the day of sensitization, then every second day for a total of eight injections/rat. Control immunized rats were treated in an identical manner with saline. On day 14, all

animals were injected intradermally with 30 µg of BSA and a 1:10 dilution of old Tuberculin (OT). Arthus reactions were read at 4 hours and delayed reactions at 24 hours. Rats treated with Met-Enk and Leu-Enk showed an appreciable dimunition of hypersensitivity skin reaction to BSA and OT. Grossly, the degree of edema (Arthus reaction) and induration (delayed reaction) were sharply reduced. Histologically, the infiltration of the dermis with polymorphonuclear (Arthus reaction) and mononuclear cells (delayed) reaction) was poor compared with the massive cellular infiltration seen in skin reations of saline-treated controls.

In an additional experiment, Enk-treated Wistar rats were implanted under the kidney capsule with the thyroid from Lewis rats. Microscopically, many of the thyroid grafts in Enk-treated recipients showed little evidence of rejection at 3 and 4 days. However, grafts in saline-treated rats exhibited an advanced rejection. Suppression of hypersensitivity skin reactions and rejection of thyroid allografts was more pronounced in rats treated with Met-Enk than in rats given Leu-Enk. Thus, these results demonstrate a depressed cell-mediated immune responsiveness in rats treated with Enks. The studies of humoral immunity and cellular immunity strongly indicate that enkephalins may be important regulators of immune mec' isms *in vivo*.

Role of Endogenous Opiates in the Pathogenesis of Fatal Anaphylactic Shock

Research was described by S. Amir (Department of Isotope Research, The Weizmann Institute of Science, Rehovot, Israel) providing new evidence that endogenous opiates play a central role in the pathogenesis of fatal anaphylactic shocks. Endorphins, which are released in shock, appear to exert their effect by acting through CNS receptors to retard peripheral physiologic compensatory processes associated with activation of the sympathoadrenal-medullary-beta-adrenoceptive system.

The basis for Amir's studies was that endorphins are known to affect the course of circulatory shock and had been implicated in anaphylatic shock, suggesting that opiate antagonists, including those with receptor level actions such as naloxone and naltrexone, and compounds such as thyrotropin-releasing hormone (TRH) may have significant therapeutic value in treating this condition.

In Amir's studies, immunized mice were treated systemically with doses of naltrexone or naloxone and subsequently challenged with a lethal dose of antigen. Both naloxone and naltrexone were found to significantly improve survival in the shocked mice and reversed the hypoactivity induced in immunized mice by a sublethal dose of the sensitizing antigen. In contrast, treatment with naltrexone methyl bromide, an opiate antagonist which is impermeable to the CNS, was ineffective in reversing the hyperactivity or in improving survival. This suggests that naloxone and naltrexone exert their antianaphylactic actions largely by blocking the effects of endorphin in the CNS. In another series of studies, the mechanism of action of naloxone in improving survival in anaphylactic shock was investigated. Pharmacological or surgical treatments which impair the functional integrity of the sympathoadrenal-medullary-beta-adrenoreceptive system reverse the beneficial effect of systemically or centrally administered naloxone. These findings suggested that naloxone may exert its antianaphylactic effect by acting in the CNS to improve peripheral physiologic compensatory responses associated with activation of the sympathoadrenal-medullary-beta-adrenoreceptive system. In another series of experiments, Amir found that systemic or intracerebral injections of TRH significantly improved the survival rate in shocked mice. TRH, like naloxone, appeared to exert its effect by acting centrally to selectively enhance outflow to beta-adrenoreceptive sites.

Enhancement of Host Resistance to Viral and Tumor Challenge

R.E. Faith, A.J. Murgo, C.N. Clinkscales, and N.P. Plotnikoff (University of Houston, Houston, Texas, West Virginia University Medical Center, Morgantown,

and Oral Roberts University School of Medicine, Tulsa, Oklahoma) presented their work on the enhancement of host resistance to viral and tumor challenge by treatment with Met-Enk. Host resistance to disease is dependent upon a number of factors. Recent evidence indicates that NK cells play an important role in resistance to both neoplastic and virally induced disease. Faith et al. treated C57BL/6 mice with Met-Enk (1, 3, or 10 mg/kg b.w.) and found significant increases in NK activity of splenic lymphocytes 24 hours following injection of the Enk. Enk treatment was also found to enhance host resistance. The short-term survival of A/J female mice following 4 SV-2 infection was significantly increased by daily subcutaneous injections (3 mg/kg b.w.) of Met-Enk. Similarly, daily doses of 50 µg of Met-Enk for 7 or 14 days inhibited the local subcutaneous tumor growth of B16-BL6 melanoma in C57BL/6 mice.

Lymphocyte Production of Endorphin

E.M. Smith, D. Harbour-McMenamin, and J.E. Blalock (Departments of Psychiatry and Microbiology, University of Texas Medical Branch, Galveston, and Department of Physiology and Biophysics, University of Alabama at Birmingham) presented their studies dealing with lymphocyte production of endorphin. Many stimuli will induce leukocytes to synthesize opiate-related peptides. Newcastle disease virus was the prototype inducer, but Herpes simplex virus and the B-lymphocyte mitogen, bacterial lipopolysaccharide (LPS) have subsequently been shown also to be effective. When infected with virus, both B- and T-lymphocytes synthesize biologically active endorphin-like peptides. Nonviral inducers cause only a fraction of the lymphocytes to synthesize ACTH and endorphins. Therefore, Smith et al. have been trying to identify the specific subsets of lymphocytes responding to the different stimuli.

The B-lymphocyte mitogen LPS was used to stimulate fractionated mouse splenocytes in order to identify the major population of endorphin-producing cells. Smith et al. found that 47 percent of LPS-treated IgM-bearing splenocytes stained positive with an antiserum specific for γ-endorphin. In contrast, Thy 1.2-bearing sphenocytes exhibited only background staining. A subpopulation of LPS-stimulated macrophages also stained positive for endorphin by immuno-fluorescence. Purification by antibody affinity chromatography and structural characterization of the immunoreactive (ir) endorphin by gel filtration showed it to be the same size as α- or γ-endorphine (1800 daltons). In the past, Smith et al. had found that ir endorphins would block the binding of dihydromorphine to opiate receptors on mouse brain preparations. More specific characterization of the binding in this study showed the LPS-induced ir endorphin bound to delta-type, opiate receptors. The ir endorphin bound specifically to mouse NG 108 cells, a neuroblastoma/glioma cell line which carries only delta-type opiate receptors. Thus, these results suggest that the endogenous opiate induced during endotoxic shock may originate from B-lymphocytes and macrophages. This is further evidence in support of a regulatory interaction between the immune and neuroendocrine systems operating through common signal molecules and receptors.

Effects of Endorphins on the Generation of Antibody Response

J.C. Heijnen, C. Bevers, A. Woudenberg, J. Ziljstral, and R.E. Ballieux (The Netherlands) reported on investigations to study the modulatory effects of endorphins on the generation of the antibody response in vitro of human peripheral blood B cells as well as to demonstrate one of the cellular mechanisms by which endorphins may influence lymphocyte reactivity.

In order to detect a modulatory effect of endorphins on the antibody response, human peripheral blood cells were cultured with a primary antigen ovalbumin (OA) and a secondary antigen, tetanus toxoid (TT) in the presence of various concentrations of α- or β-endorphin (αE or βE). Heijnen et al. found that αE can

inhibit the primary (IgM) anti-OA response as well as the secondary (IgG) anti-TT response. βE, however, is capable of enhancing the primary anti-OA response, whereas βE can either enhance or inhibit the secondary anti-TT response.

To investigate the cellular mechanisms by which endorphins may modulate the immune response, Heijnen et al. studied the effect of endorphins on the fluidity of lymphocyte membranes. They found that endorphins can influence the fluidity of the lymphocyte membranes which may have functional consequences for the expression of various functionally important receptors on the cell surface, leading to an altered immune response.

4 NEUROENDOCRINE RECEPTORS IN THE IMMUNE SYSTEM

Interactions of Immune and Neuroendocrine Systems

E.M. Smith (Department of Psychiatry and Behavioral Sciences and Microbiology, University of Texas Medical Branch, Galveston, Texas) presented studies from his laboratory dealing with interactions between the immune and neuroendocrine systems. He discussed his findings as well as those from other laboratories on the concept of a molecular basis for interactions between the immune and neuroendocrine systems.

Leukocytes stimulated with endotoxin or viruses or with the hypothalamic releasing hormone, corticotropin-releasing factor synthesize the POMC-derived peptides, ACTH and endorphins. Similar to pituitary POMC peptides, the synthetic glucocorticoid dexamethasone suppresses such production. Thus, the POMC gene appears not only to be expressed and processed by cells of the immune system but is also controlled by brain and adrenal hormones in a fashion analagous to that observed in the pituitary gland. Other stimuli induce subpopulations of lymphocytes to produce other neuroendocrine hormones including thyrotropin, chorionic gonadotropin, vasoactive intestinal peptide, somatostatin, and growth hormone. These neurohormones can act as immunoregulators. They function by binding to specific receptors on the lymphocytes that appear structurally identical to corresponding receptors on endocrine tissue. Thus, it appears that leukocytes produce and are acted upon by neuroendocrine peptide hormones. According to Smith, such findings suggest a complete regulatory circuit between the immune and neuroendocrine receptors which operates through a common set of peptide hormones and their receptors. On a molecular basis, Smith thinks that these observations may explain the effects of the CNS on immune functions as well as the alteration in host homeostasis and psychology that occur during infection and neoplasia.

Neuropeptide Receptors in Lymphatic Tissue Cells

C.J. Wiederman and C.B. Pert (Section on Brain Biochemistry, NIMH, Bethesda, Maryland) studied the presence of neuropeptide receptors on cells in lymphatic tissues by autoradiographic visualization of neuropeptide receptors in rat spleen. Neuropeptides are short signal peptides found in the nervous system as well as cells which originate in gut and bone marrow. Their signal specificity resides in distinct classes of recognition molecules that are also found in the brain and body, thus joining the brain, glands, and immune system in a network of communication.

In previous studies by Wiederman and Pert as well as by other researchers, specific receptor-mediated interactions of neuropeptides with immune and blood cells had been defined pharmacologically and by direct analysis of binding to circulating blood cells. In the present study Wiederman and Pert carried out a morphological investigation of neuropeptide receptors on spleen sections. Certain neuropeptides including substance P (SP), somatostatin, cholecystokinin, and vasopression were shown to bind specifically with appropriate displacement by a series of peptide analogs to morphologically defined spleen areas that are known to represent the presence of different cell types (i.e., periarteriolar

sheath, germinal center, marginal zone, and red pulp). For example, the SP receptor was found to be located throughout the red pulp (macrophages) and in some of the germinal centers of the white pulp (B-lymphocytes). A much higher concentration of SP receptors was found in the marginal zone (B-lymphocytes). Almost no SP could be demonstrated in the periarteriolar sheet (T-lymphocytes).

5 NATURAL MEDIATORS AND PHARMACOLOGY OF NEUROIMMUNOMODULATION

Peptide Regulation of Lymphocyte and Mast Cell Function

D.G. Payan, J.P. McGillis, F.K. Renold, T. Chernov-Rogan, M.L. Organist, and E.J. Goetzl (Howard Hughes Medical Institute and Departments of Medicine and Microbiology, University of California Medical Center, San Francisco, California) reviewed and discussed the regulation of lymphocyte and mast cell function by peptides of the sensory nervous system. Their conclusion was that neuropeptides and immunologically derived neuropeptide-like factors appear to mediate bidirectional communication between the nervous and immune system that is critical for optimal host defense and expression of hypersensitivity.

Peptides of primary afferent nerves regulate the development, tissue distribution, and function of diverse cellular constituents of immediate and delayed immunological reactions. SP was found to elicit mediator release by mast cells with a greater potency for mucosal than connective tissue mast cells. It attracts polymorphonuclear leukocytes and macrophages and stimulates T-lymphocytes. Stereospecific receptors for SP and other neuropeptides which tranduce the observed effects on function of different subsets of mouse and human T-lymphocytes were defined by flow cytometry with fluorescently labeled peptides and analysis of the binding of radiolabeled peptides. Payan et al. isolated human B-lymphoblast receptors for SP for structural and immunochemical analyses. They found that basophiles, mast cells, and macrophages contain peptides that are antigenically and immunofunctionally similar to SOM and SP in an apparent weight ratio of 5 to 10:1. The amino acid sequences of the SOM-like factors purified from extracts of rat leukemic basophils revealed both homologies and differences from those of neuroendocrine SOMs.

Suppression of Immune Response by Drugs

Suppression of the immune response by drugs interfering with a metabolism of serotonin was studied by M. Boranic, D. Pericic, M. Poljak-Blazi, V. Sverko, and T. Marott (Rudjer Boskovic Institute, Department of Experimental Biology and Medicine, Zagreb, Yugoslavia). The work was based on the assumption that neurohumoral control of the immune response, particularly in stressed animals, involves central serotonergic mechanisms. Rats immunized with sheep erythrocytes were stressed by repeated restraints and treated with a precursor of serotonin (5-hydroxytryptophan, 5-HTP) or with an inhibitor of serotonin synthesis (parachlorophenylalanine (PCPA)). As expected, repeated stresses reduced the plaque-forming cell (PFC) response. Treatment with 5-HTP also reduced the PFC response and was potentiated by the immunosuppressive effect of stress. This was accompanied by increased metabolism of serotonin in the brain, as indicated by increased concentration of its metabolite, 5-hydroxytrytotophan, 5-HIAA) in cerebrale tissue. Treatment with PCPA also suppressed the PFC response, but this suppression was accompanied by decreased levels of brain serotonin and of 5-HIAA. Plasma corticosterone levels were elevated in rats treated with PCPA. Incubation of peripheral blood lymphocytes with 5-HTP or PCPA reduced the *in vitro* PFC response to sheep erythrocytes in a dose-dependent manner. Thus, it seems that putative central effects of PCPA on serotonergic regulation of the immune response were outweighed by its effects on corticosterone secretion and on lymphoid cells.

6 NEUROPHYSIOLOGICAL CORRELATES OF NEUROIMMUNOMODULATION

Neurophysiological Correlate of an Immune Response

D. Saphier, O. Abramsky, S. Feldman, G. Mor, and H. Ovadia (Department of Neurology, Hadassah University Hospital, Jerusalem, Israel) presented their work on a neurophysiological correlate of an immune response. Certain regions of the CNS, including the prepoptic area (PO) have been shown to influence the course of some immune responses. Such influences probably depend on humoral chemical signals generated during the course of immune responses and modulating neuroendocrine regulatory mechanisms in the CNS. In an attempt to examine possible neurophysiological changes during the course of the immune response to intraperitoneal injection of sheep red blood cells (SRBC) Saphier et al. recorded daily changes in POA and hypothalamic paraventricular nucleus (PV) multiunit activity (MUA). Male rats bearing chronically implanted recording electrodes showed increases of up to 300 percent in POA MUA on day 5 following SRBC immunization, with significant decreases being recorded on days 3 and 8. PVN MUA recorded from the site of corticotrophin-releasing factor (CRF) synthesizing cells revealed significant increases on day 6 after the injection. These results appear to correlate with the course of antibody production during the immune response and with concommitant elevation of corticosterone levels, secretion of which is controlled by hypothalamic CRF.

Influence of Biological Rhythms

B. Radosevic-Stasic, L. Polic, and D. Rukavina (Department of Physiology and Immunology, Medical Faculty, Rijeka, Yugoslavia) investigated the antibody and cell-dependent immunity of rats sensitized at different times of the day and the consequence of the blockade of the sympathetic nervous system on these events. As many other systems, the immune system is also characterized by time-dependent cyclicity of its functions. Although these biological rhythms are presumably endogenous and genetically fixed, the role of certain hormones and neurotransmitters in the synchronization has been suggested.

For their studies Radosevic-Stasic et al. followed plaque-forming cells (PFC) and dynamics of local group versus host reaction (GVHR) after the injection of SRBC in intact rats and rats treated with Labetalol, which blocks α- and β-adrenergic receptors. Control groups were treated with saline. It was found that Labetalol did not influence the humoral immune response in comparison with simultaneously treated controls. However, sympathectomy was found to produce a reversion of the normal circadian rhythm which existed in PFC formation, i.e., the same dose of SRBC applied at 5:00 p.m. produced a significantly greater number of PFC than when injected at 8:00 a.m., while Labetalol resulted in an opposite finding. Similar changes dependent on the timing of the treatment with Labetalol were observed in the dynamics of local GVHR. The results point to the existence of circadian rhythms in the immune functions and to the role of the sympathetic nervous system in their formation.

Muramyl Peptides as Physiological Modulators

J.W. Karaszewski and J.M. Krueger (Department of Physiology, University of Tennessee, Memphis, Tennessee) investigated muranyl peptides as possible mammalian physiological modulators. Muramyl peptides (MP) have been implicated in several biological processes including immune responses as well as temperature and sleep regulation. However, there are no known mammalian synthetic pathways for some of the components of MPs (e.g., muramic acid [MA] and diaminopimelic acid [DAP]). Thus it has been postulated that MPs are vitamin-like. If MPs are involved in normal mammalian CNS and immune processes, one would expect to find MPs present in these and other tissues as well as the existence of transport systems to allow and control entry of MPs into brain from blood. Therefore, these researchers developed tissue extraction

and quantitation methods to study these questions. Reverse-phase high-performance liquid chromatography (HPLC) with a C18 column and precolumn derivitization with phenylisothiocyanate allowed measurement of MA and DAP over the range of 4-3600 picomoles. Karaszewski and Krueger found that MA is present in mammalian tissue. Also, they determined brain clearance from blood of a radiolabeled monoiodinated MDP-TyrOMe maintained at constant arterial concentration. They found that a carrier system (i.e., MDP-reduced MDP-TyrOMe clearance) for transporting MPs from blood to brain does exist. These observations are consistent with the hypothesis of Karaszewski and Krueger that MPs are integrated into normal mammalian physiological processes.

7 STRESS AND IMMUNITY

Effects of Psychogenic Stress

H.K. Fischman, D. Kelly, and R. Pero (New York State Psychiatric Institute, New York) studied the effect of psychogenic stresses on DNA damage. Exposure of an intact organism to a variety of behavioral stressors can trigger an increase in Sister Chromatid Exchanges (SCEs) within 24 hours. Within 2 hours of exposure there is also an increase in DNA repair. This is a property shown by a diverse range of stresses and appears to be graded with respect to severity. In the study of Fischman et al., rats were exposed to stress in either cold or warm water or to no stress. Two hours later a bromodeopuridine pellet was implanted subcutaneously. Sacrifice of rats occurred 25 hours post stress and the bone marrow was analyzed for SCEs. Both warm and cold swims were found to significantly elevate SCEs. The generality of the phenomena was also tested in rats exposed to either: cold swims; white noise; intermittent, inescapable footshock (IFS); continuous IFS; or no stress. Blood was collected 2 hours later and unscheduled DNA synthesis (UDS) measured in leukocytes. The level of UDS in stressed rats was found to be twice that of controls. In another experiment, rats received either 480, 240, or 120 shocks, or were placed in the same chamber where other rats had been shocked, or were left in their home cages. SCEs and chromosome alterations were related in a graded manner to the severity of IFS. Rats exposed only to the physical environment in which conspecifics had been stressed showed significant intermediate elevations. UDS measurements produced similar, graded results. Fischman et al. proposed a hypothesis based on the above results that psychogenic stress may trigger pathology by altering DNA.

Emotional Stress and the Immune System

R.E. Ballieux, G. Croiset, H.D. Veldhuis, D. de Wied, F. Berkenbosch, B. Bohus, and C.J. Heijnen (Department of Clinical Immunology, University Hospital, Utrecht, The Netherlands) reported on their studies of the influence of various forms of emotional stress on the immune system. The aim of their study was to investigate whether different forms of emotional stress have their own characteristic way of modulating the immune response of male rats. Ballieux et al. studied the influence of three types of acute stress: the one-trial learning passive avoidance test; the stress induced by a mild restraint; and the stress induced by forced swimming. The rejection of the immune system was tested by determining the proliferative response of rat spleen cells and peripheral blood lymphocytes as well as on the capacity to generate an antibody response *in vivo*. The results showed that the various forms of stress modulate the immune system in a different way, either by enhancing or by inhibiting the immune response.

Ballieux et al. also investigated the influence of social stress induced in rats by living in a colony in a stable hierarchy. The effect of this form of (chronic) stress was analyzed by determining the proliferative response of lymphocytes of the dominant, the subdominant, as well as of the submissive rats. The animals were also studied with regard to the histopathology of various organs. The results showed that the modulation of the immune response is an inherent

characteristic of the position in the social hierarchy.

Effects of Diazepam

D. Pericic, H. Manev, M. Boranic, M. Pojak-Blazi, and N. Lakic (Rudjer Boskovic Institute, Department of Experimental Biology and Medicine, Zagreb, Yugoslavia) spoke about their studies on the effect of diazepam on brain neurotransmitters, plasma corticosterone and the immune system of stressed rats. Drugs interfering with neurotransmission often influence the immune system. In this study, Pericic et al. wanted to find out whether and how diazepam, a widely used anxiolytic drug which primarily potentiates transmission mediated by γ-aminobutyic acid (GABA) but also changes the activity of other neurotransmitter systems, affects the immune system. Rats immunized with sheep erythrocytes were stressed by immobilization (3 hours daily) and/or treated with diazepam (1 or 10 mg/kg) for 4 consecutive days. Pericic et al. found that the activity of hypothalamic glutamate decarboxylase (the enzyme taking part in GABA synthesis) was not affected by either of these treatments. The levels of brain noradrenaline decreased in all treated groups while the level of dopamine decreased only in stressed groups treated with diazepam (1 mg/kg). Although both treatments (stress and diazepam) enhanced the turnover of 5-hydroxytramine (5-HT) in the brain, treatment with both doses of diazepam prevented the stress-induced rise of 5-HT turnover. The levels of plasma corticosterone were enhanced in all stressed rats (with and without the drug) but also in rats treated with diazepam, 10 mg/kg. This finding was in accordance with the enhanced weights of adrenal glands in the same group of rats. Since the PFC response was reduced in all stressed animals and in animals treated with diazepam (10 mg/kg), Pericic et al. believe that only higher doses of diazepam induce immunosuppression. This finding correlates with the diazepam-induced enhanced secretion of glucocorticoids but not with the metabolism of GABA and 5-HT or with the levels of brain noradrenaline and dopamine.

Changes in Population of T-Cell Subsets

A. Teshima, H. Sogawa, H. Kihara, S. Nagata, and Y. Ago (University of Kyushu, Fukuoka, Japan) investigated changes in the population of T-cell subsets in stressed mice. It is well known that stress induces atrophy of the thymic gland where T-cells proliferate and grow and that stress influences the functions of T-cells; e.g., cytotoxicity. In their study, Teshima et al. investigated the influence of stress on populations of subsets of T-cells in the blood, the thymus, and the spleen using flow cytometry and monoclonal antibodies (Mabs) to measure Lyt 1, Lyt 2, and Thy 1.2. Female mice 5 to 8 weeks old (C57 Black and C3H) were used with immobilization stress being given for 20 hours per day. The results showed changes in the percentages of the populations of Lyt 1, Lyt 2, and Thy 1.2 cells after immobilization stress. Percentages of Lyt 1 and Thy 1.2-positive cells in the blood increased after 3 days of stress and that of Lyt 2 cells after 2 days of stress. In the thymus, Lyt 1 and Lyt 2-positive cells increased on the first day after stress, and in the spleen, Lyt 1 and Lyt 2-positive cells decreased on the first day after stress. However, such changes caused by stress were prevented by pretreatment with diazepam. These results show that stress influences immune cells through the agency of the CNS.

8 SLEEP AND IMMUNE FUNCTIONS

Unfortunately three of the five presentations scheduled for this session were cancelled so that only the two available talks are reported here.

Immune Modulators as Sleep Promoters

J.M. Krueger (Department of Physiology, University of Tennessee, Memphis) spoke about his research implicating immune modulators as sleep promoters. Krueger said that the search for endogenous sleep-promoting factors originated from the observation that prolonged wakefulness leads to a desire to sleep. He has characterized an endogenous sleep-promoting factor, termed Factor S(FS), obtained from brain and urine as a muramyl

peptide (MP). Although MPs are constituents of mammalian tissue, Krueger thinks that they are probably of bacterial origin. Therefore, he examined other bacterial products and certain leukocyte products for sleep-promoting activity. Krueger found that MPs, lipid A (LA), interleukin-1 (IL-1), tumor necrosis factor (TNF), and alpha-2 interferon (IFN) all were able to enhance slow-wave sleep (SWS). Their effects on rapid eye movement (REM) were more variable, although inhibition of REM was usually observed. Each of these substances can also induce fever, although fever responses can be blocked without affecting sleep response. His research suggests that these substances may be involved in regulation of normal sleep because:

1. Sleep and waking behaviors after IL-1, IFN, TFN, and MP treatment are normal.
2. MPs, IL-1, IFN, and LA enhance amplitudes of EEG slow waves; similar supranormal slow waves are observed after sleep deprivation.
3. Brain temperature changes that are tightly coupled to sleep stages persist in animals treated with IL-1, IFN, TFN, LA, and MP.
4. An IL-1 antagonist, α-MSA inhibits SWS and REM sleep.

Krueger thinks that the finding that IL-1, IFN, MPs, TFN, and LA enhance SWS suggests that the sleepiness that often occurs during infectious diseases may be due to their CNS actions. He also believes that his results indicate that sleep may play an important role in recuperative processes whether it is recovery from a day's activity or from damage induced by a disease.

Muramyl Dipeptide as a Sleep Promoter

K. Masek (Institute of Pharmacology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia) also investigated the sleep-promoting activity of muramyl dipeptide (MDP) with the aim of finding out the involvement of certain brain structures in this activity. With the aid of electrolytic lesions as well as with the use of biochemical and pharmacological approaches, Masek observed that the serotonergic system seemed to be involved and has identified certain serotonergic group cells of the brainstem in the sleep promoting activity of MDP. Masek also tested the possibility of a direct action of MDP with the serotonergic system of peripheral tissues and concluded that MDP behaves as a partial serotonergic agonist. The possible interaction of MDP with serotonergic receptors was confirmed by direct binding studies with radiolabeled compounds.

9 BEHAVIOR, ASSOCIATIVE LEARNING, AND MEMORY

The Behavioral Modulation of Immunity

R. Ader (University of Rochester School of Medicine and Dentistry, Rochester, New York) presented a review of his work as well as that of other researchers on behavioral modulation of immunity.

Behavioral factors, including the experimental history of the organism, current social and environmental conditions, and so-called "stress" are capable of influencing the immune system, as evidenced by the exacerbation of immunologically mediated disease states and by the direct measurement of various parameters of immune function. Involvement of the CNS in the modulation of immunity is most dramatically illustrated by the classically conditioned alteration of immune responses.

Exposing animals to a conditioned stimulus (CS) previously paired with an immunosuppressive drug results in an attenuation of the antibody and the plaque-forming cell response to T-cell dependent and independent antigens and an attenuation of cell-mediated responses. Unreinforced exposures to the CSs result in extinction of the conditioned response. The results from Ader's laboratory as well as other laboratories demonstrate that conditional alterations in immunologic reactivity are not confined to the one-trial taste aversion paradigm or the use of immunopharmacologic agents as unconditional stimuli. Still other data

suggest that a net enhancement of immunologic reactivity can be conditioned.

The biologic impact of conditioned changes in immune responses is illustrated by studies in which the onset and treatment of autoimmune disease, adjuvant-induced arthritis, and mortality to a transplanted tumor were altered by reexposing conditioned animals to the CS previously paired with an immunomodulatory agent. Preliminary data also suggest that the disregulation of immune function characteristic of mice with lupus is reflected in their capacity to acquire conditioned responses based on the effects of an immunosuppressive drug.

Conditioning: Immunoenhancing Effects of CY

A study on the conditioned enhancement of delayed-type hypersensitivity (DH) in mice was presented by Nicholas Cohen and Robert Ader (University of Rochester Medical Center, Rochester, New York) and D. Bovbjerg (Cornell University Medical College, New York). These researchers designed experiments to determine whether the immunoenhancing effects of cyclophosphamide (CY) could be conditioned. CY is best known as an immunosuppressive drug. However, depending on the dose and timing relative to sensitization and challenge with antigens, CY can also enhance immune responses (probably by suppressing regulatory suppressor systems). It had been shown previously by Ader and others that the immunosuppressive effects of CY could be classically conditioned.

In the study of Cohen et al., mice were conditioned by pairing their consumption of a saccharin solution (CS) with injection of CY on each of 3 consecutive days. Three weeks later, these conditioned animals were sensitized with SRBC. They were then reexposed to the CS on days 2, 3, and 4 after sensitization. On days 4, 11, and 18, the mice were challenged with SRBC, and the DH reaction (footpad swelling) was measured at 24 and 48 hours.

Cohen et al. found that the DH responses of conditioned mice were significantly enhanced on days 11 and 18 when compared with either nonconditioned animals subjected to the same protocol or conditioned animals not reexposed to the CS. Thus, the results showed that the apparently immunoenhancing as well as manifestly immunosuppressive consequences of CY can be classically conditioned.

Regulation of Natural Immunity by Conditioning

R.N. Hiramoto and V.K. Ghanta (Department of Microbiology and Biology, University of Alabama, Birmingham) presented a study on the regulation of natural immunity (NK activity) by conditioning. They used a combination of saccharin and LiCl (CS) along with the unconditioned stimulus (US), poly I:C. In another study, Hiramoto and Ghanta used the combination of cyclophosphamide as the US. They observed that the combination of saccharin-LiCl treatment with poly I:C conditioned the NK response in a positive way. The use of cyclophosphamide conditioned the animals' NK response in a suppressive way. The duration of conditioning was short, as only one association trial was used. The short duration required will allow these researchers to study the mechanism of conditioning.

Conditioned Taste Aversion

V.J. Djuric, B.M. Markovic, M. Lazarevic, and B.D. Jankovic (Institute for Psychology and Immunology Research Center, University of Belgrade, Yugoslavia) reported on a study of conditioned taste aversion in rats subjected to anaphylactic shock. Rats readily learn to associate illness with ingestional stimuli and show taste aversion (TA) to otherwise prefered flavor if it was previously used as a signal for the illness-inducing unconditioned stimulus (US). Thus far, ionizing radiation and various pharmacological substances have been employed by Djuric and others as aversive stimuli in the TA paradigm. In the present study, the objective was to examine whether anaphylactic shock can be used as an effective US. For this purpose, outbred female Wistar rats were sensitized to ovalbumin: 3 subcutaneous injections (10 mg of albumin in aluminum hydroxide gel)

were given at 2-week intervals. Sixteen days after the third injection of ovalbumin conditioned stimulus (0.125 percent of sodium saccharin) was immediately followed by a nonlethal anaphylactic shock provoked by an intraperitoneal injection of 1 mg or 2 mg of ovalbumin. Twenty-four or 96 hours later a two-bottle preference test, tap water versus 0.125 percent of saccharin solution was carried out for 7 consecutive days. All of the groups that drank saccharin prior to the induction of shock, showed marked behavioral avoidance of the flavor except the group that was preexposed to it (the latent-inhibition group which was given saccharin solution for 5 days before the induction of shock). These results suggest that rats can associate gustatory stimulation with anaphylactic shock and its consequences.

Suppression of Plaque-Forming Cell Responses

D. Bovbjerg, Y.T. Kim, G.W. Siskind, and M.E. Weksler (Department of Medicine, Cornell University Medical College, New York) presented a study on the suppression of plaque-forming cell responses by classical conditioning with cyclophosphamide. Mice were allowed to drink saccharin-flavored water (Sac) immediately before they were injected with cyclophosphamide (Cy), a procedure designed to establish an association between the Sac consumption and the physiological affects of Cy in a classical conditioning experiment. Control, nonconditioned mice were similarly injected with Cy but were first exposed to Sac 1 week later. Two weeks after the Cy injection (on day 0) all mice were immunized with trinitro-phenylated sheep red blood cells (TNP-SRBC) and then were reexposed to the Sac (the conditioned stimulus) on days 0, 2, and 4. Bovbjerg et al. found that conditioned mice (CS group) had significantly decreased numbers of anti TNP-SRBC plaque-forming cells (PFC) on day 5 compared to nonconditioned mice. Thus, Bovbjerg et al. think that one mechanism for the reductions in antibody levels induced by conditioning may be a reduction in the number of antibody-producing cells in the spleen after reexposure to the conditioned stimulus Sac.

Psychopharmacological Activity of Immune Complexes

N. Schupf and C.A. Williams (Department of Psychology, Manhattanville College and Division of Natural Science, State University of New York, Purchase, New York) presented their work on the psychopharmacological activity of immune complexes in rat brain. They had previously found that injection of immune complex-forming systems (ICS) via implanted cannulae to the periformical hypothalamus stimulates eating in sated rats and increases the eating response to norepinephrine (NE). The ICS effect is associated with aggregation of polymorphonuclear cells at the injection site, suggesting activity of leucotoxins and the possibility that the complement cascade had been activated with the production of anaphylatoxins C3a and C5a. In the present study, Schupf and Williams tested the possibility that the ICS effect was mediated by complement-derived peptides by injecting C3a and C5a directly to the perifornical site. They found that C3a potentiated NE induced eating and C5a induced eating in sated rats, an effect which mimics that of focal NE. The induction of eating by both NE and C5a was blocked by the α-adrenergic antagonist, phentolamine. If the anaphylatoxins or other by-products of the complement cascade were responsible for the immune complex effect, interference with the initiation of the cascade or with the conversions of C3 to C3a or C3b should abolish the behavioral response.

Two approaches were taken to show that activation of the complement cascade was required for the ICS effect:

1. The effect of treatment with rabbit antihuman serum albumin (aHSA:3 µg) followed in 30 minutes by a 20-fold excess of antigen (HSA:10 µg) was compared to the effect of affinity-purified aHSA treated with pepsin to produce the divalent (therefore complex-forming) non-complement-fixing IgG fragment $F(ab')_2$.

2. Goat antibody against the third component of complement (aC3:0.15 µg) was

injected to the perifornical site before the ICS injections were made.

Schupf and Williams found that immune complexes formed with the noncomplement-fixing $F(ab')_2$ fragment of the rabbit aHSA did not induce eating. Also, the normally active IgG antibody complexes did not induce eating if the site had been pretreated with a C3. However, the ability of the animals to respond to NE (19 nmol) or to C5a (5 pmol) was not impaired by aC3. Schupf and Williams therefore concluded that the ICS effect depends upon focal complement activation and that production of anaphylatoxins may interfere with catecholomine function.

10 CANCER, AIDS, AND IMMUNITY

Immunomodulatory Effects of Enkephalins

N.P. Plotnikoff, G.C. Miller, N. Nimeh, R.E. Faith, A.J. Murgo, and J. Wybran (Oral Roberts University; Immunodiagnostics Inc., Tulsa, Oklahoma; The Cleo Craig Memorial Cancer Foundation, Lauton, Oklahoma, and Hopital Erasme, Free University of Brussels, Belgium) carried out a collaborative study to test the immunomodulatory effects of enkephalins in human subjects. This study was based on previous work by these groups and others that in experimental animal studies the enkephalins and, in part, the endorphins can stimulate the cellular components of the immune system.

In one study with normal volunteers, the immunomodulatory effects of methionine enkephalin in a wide range of doses (one to 250 μg/kg) was investigated. No overt toxicity was observed. Significant increases in all T-cell subsets, lymphocytic proliferation, and NK cell activity were found. In cancer patients, including AIDS, similar increases in T-cell subsets and lymphocyte proliferation were also observed. Furthermore, significant increases in interleukin-2 receptor expression and levels were found; as well as increases in IL-1 and γ-interferon and activation of macrophages were also observed. Not only were there significant increases in all T-cell subset parameters in two patients with Kaposis Sarcoma (AIDS) treated with met-endekhalin in the range of 10 to 25 μg/kg, but the sarcoma lesions were observed to be crusting, healing, and diminishing in size. Lymph nodes in the axillary area were palpably smaller. Similar findings were observed in patients with lung cancer receiving an infusion of met-enkaphalin in a dose range of 10 to 25 μg/kg. Based on all these results, Plotnikoff et al. believe that met-enkaphalin may have therapeutic potential as an immunomodulator agent.

Modulation of Macrophage Function by Neuropeptides

R. Peck (Central Research Units, F. Hoffman LaRoche & Co. Ltd., Basel, Switzerland) spoke about the work of his group on the modulation of macrophage function by neuropeptides. The immune system and the neuroendocrine system affect each other via molecules and recepters shared by both systems. Neuropeptides or neurohormones may act either positively or negatively in regulating the activities of a key cell of the immune system, the macrophage. Adrenocorticotropic hormone (ACTH) and somatostatin block the tumoricidal activity of macrophages induced by recombinant gamma interferon (γ-IFN), an immunomodulating lymphokine. In contrast, substance P (SP) was found to increase tumoricidal activity, both independent of γ-IFN and in addition to γIFN. Neurotensin, α-endorphin, β-endorphin, met-enkephalin, vasopressin, and substance P did not affect tumoricidal function, either alone or in combination with γ-IFN. SP, but not the other neuropeptides, increased substantially the proportion of macrophages producing superoxide anions. According to Peck, this evidence suggests a possible influence of SP on macrophage capacity to deal with microbial infection. Such positive and negative modulation of macrophage effector functions could contribute to the influence of cognitive stimuli (physical, emotional, chemical) in infection and neoplasia (cancer).

Immunological Effects of Met-Enkephalin

J. Wybran, L. Schandené, and G. Vandermoten (Departments of Immunology and Pneumology, Hospital Erasme, Free

University of Brussels, Belgium) presented studies on the immunological effects of the administration of met-enkephaline (Met-Enk) to lung cancer patients. Met-Enk was administered intravenously at a dose of 50 µg/kg or 100 µg/kg to seven patients with lung cancer. These patients were newly diagnosed and without prior surgery, chemotherapy, radiotherapy, or immunotherapy. Some immunological tests were performed before and after Met-Enk administration (2 hours, 4 hours, and day 6 post injection). The results were as follows: no toxic effects were observed. Out of seven patients, four showed an increase in the percentage of blood-active T rosettes, five in the percentage of OKT 10 blood lymphocytes, seven in the percentage of Leu 11 blood positive cells (more than twice the initial value in four of the patients). In these seven patients, five showed an enhancement in NK activity (in three, the NK activity increased by 100 percent). These results indicate that Met-Enk very rapidly modifies the immunity of cancer patients and that a remarkable increase of NK activity can be achieved with Met-Enk. In addition to confirming *in vitro* data from Wybrans' group as well as others, these results, suggest, according to Wybran, that Met-Enk could be used an an immunomodulating agent and as a modifier of the biological response.

11 PSYCHIATRIC AND NEUROLOGICAL DISORDERS WITH IMMUNOLOGICAL FEATURES

Autoimmune Disease in Acute Schizophrenia

R. Ganguli, B. Rabin, R. Alexander, M. Lyte, U. Ragu, and R. Kelly (University of Pittsburgh School of Medicine, Pennsylvania) presented clinical and laboratory evidence of autoimmune disease in acute schizophrenia. Several reports have indicated that some patients with schizophrenia may be immunologically responding to antigens of the brain. Evidence includes the presence of activated lymphocytes in peripheral blood and delayed hypersensitivity skin reactions to brain antigen. Ganguli et al. studied a group of patients with schizophrenia to determine if they had characteristics commonly associated with the presence of autoimmune disease. The presence of antitissue antibody, activated T-lymphocytes, lymphocyte sensitized to brain antigen, and the response of lymphocytes to nonspecific mitogen were evaluated.

Thirty-one patients with well-characterized schizophrenia were studied. All of the patients were on antipsychotic medication at the time of the study. A group of normal, healthy, nonhospitalized individuals were used as the control population. Autoantibodies were detected in the serum of schizophrenic patients more frequently than in the normal control population. Twenty-five percent of the schizophrenic patients had clinical evidence for an autoimmune disease not related to their psychotic disease. Approximately two-thirds of the schizophrenic patients had activated T-lymphocytes present in the blood. In addition, 25 percent of the schizophrenic patients' peripheral blood lymphocytes were stimulated into mitosis when incubated with a saline extract of normal brain. Thus, patients with schizophrenia had evidence of activated immune systems and displayed immunologic characteristics commonly associated with the presence of an autoimmune disease. According to Ganguli et al. the data suggest that there may be a subpopulation of patients with schizophrenia who have an autoimmune process directed to the brain. Ganguli et al. are carrying out long-term follow-up studies of patients and correlation of changes in the immune system with changes in the clinical activity of disease in order to characterize the relevancy of these observations.

Production of Interleukin-2 in Schizophrenia

F. Villemain, L. Chatenoud, E. Guillibert, Y. Pélicier, and J.F. Bach (INSERM Unit 25, Hospital Necker, Paris, France) reported on finding a production of interleukin-2 (IL-2) in schizophrenia. Previous studies have suggested that immunological abnormalities may be observed in patients with schizophrenia. Several immunological parameters have been analyzed in these patients by several

researchers but the specificity of the abnormalities described have not been well defined. Villemain et al. analyzed various T-cell parameters in 10 patients with schizophrenia according to the DSM III criteria. Normal subjects as well as patients exhibiting other psychotic diseases were studied as controls. The patients analyzed were carefully selected on the basis of receiving no drugs or only one drug at the time of the study. Peripheral T-cell phenotypes as defined by the murine monoclonal antibodies OKT3 (specific for all human T-cells), OKT4 (specific for helper inducer T-cells), and OKTD (specific for suppressor/cytotoxic T-cells) were studied. B-cells were evaluated by means of immunofluorescense labeling with antihuman immunoglobulin antiserum. In addition, Villemain et al. studied the production of IL-2 (a lymphokine produced by stimulated T-cells) as a reliable parameter of T-cell function. The results obtained showed a significant decrease of IL-2 production by patients' lymphocytes as compared to controls. This decrease contrasted with the normal distribution of peripheral regulatory T-cell subsets observed in these patients.

Testing Immune Function in Psychiatric Patients

Another study of immune function in psychiatric patients was reported by M. Müller, M. Ackenheil, R. Eckstein, E. Hofschuster, and W. Mempel (University of Munich Psychiatric Hospital, Munich, West Germany). In this study, a group of 40 schizophrenic patients and a control group of 40 nonschizophrenic psychiatric patients were tested immunologically and compared with a group of 88 healthy volunteers. Immunological testing included mitogen stimulation (with Protein A, Concanavalin A, Pokeweed Mitogen, and Phythemaglutinin) and antigen stimulation (vaccinia, rubella, measles, varidase, tetanus, diptheria, tuberculin, and antigen-cocktail). Suppressor cell function was tested in mitogen systems, and in the mixed lymphocyte culture, T-cell subpopulations were determined with monoclonal antibodies. Furthermore, HLA-typing was done and cortisol levels were measured. The results showed significantly reduced activity of suppressor cells of schizophrenic patients in the pokerweed mitogen system ($p<.03$) before and after neuroleptic treatment, but also in psychiatric controls ($p<.005$). Similar results were obtained in the mixed lymphocyte culture: reduced activity of suppressor cells in schizophrenics before ($p<.0005$) and after treatment ($p<.0001$) and in psychiatric controls ($p<.01$).

Influence of Emotional State

R.N. Melmed, D. Roth, and E. Edelstein (Department of Medicine B, Child and Adult Psychiatry, Hadassah University Hospital, Jerusalem, Israel) studied the influence of emotional state on the mobilization of marginal pool leukocytes following insulin-induced hypoglycemia. In normal subjects given a standard dose of insulin (0.05 units/kg body weight) and followed with respect to their peripheral total white count, Melmed et al. observed that by 2 hours, there was mobilization of 108 percent ($n=7$) of the total cell count above the starting value. Sampling every 15 minutes over the 2-hour period showed the white cell response to be bimodal with an early peak at 45 minutes due to a rise of both polymorphonuclear leukocytes (PMN) and lymphocytes (L) and a second rise by 2 hours due only to PMNs with Ls having returned to starting values. Melmed et al. showed that these changes also occur in splenectomized patients and that the early peak follows maximal secretion of adrenalin by 15 minutes. The observed mobilization of Ls consists almost exclusively of T-cells (>90 percent) with B-cells being minimally affected. Studies using specific blockers showed the process to be mediated principally by α-receptors. Melmed et al. found that in patients who undergo the test in a state of deep relaxation, the 2-hour PMN rise is reduced by a mean of 30 percent ($p<0.05$, $n=>$). Similarly, patients suffering from major depression may show a severely reduced ability to mobilize white cells in the presence of hypoglycemia. Melmed et al. consider that these observations link emotional

changes with receptor responsivity and provide a means of analyzing in physiological terms the physical consequences of changes in emotional state.

Correlation of Ts Cells and Oligoclonal Bands in CSF

T. Pirttila, J. Antonen, R. Vikarinin, and H. Frey (Departments of Neurology and Biomedicine, University of Tampere, Finland) presented their studies on the correlation of T-lymphocyte subpopulation levels in IgG-index and to oligoclonal immunoglobulin bands in the cerebrospinal fluid (CSF) of patients with multiple sclerosis (MS). Immunological mechanisms have been reported to play an important role in the pathogenesis of MS. The intrathecal synthesis of immunoglobulins is characterized by the presence of oligoclonal bands in CSF and a high IgG index. The levels of T-lympohocytes fluctuate in peripheral blood and in CSF during the course of the disease. Suppressor T-lymphocytes (Ts) have been shown to decrease in the CSF in active MS. In the present study, 53 CSF samples (29 MS patients and 24 patients with other neurological diseases) were analyzed. Fluoresceinated Mabs were used for T-lymphocyte subpopulations and the cells were counted manually. Oligoclonal bands were demonstrated with agarose gel electrophoresis and isoelectric focusing with silver staining. A clear, positive correlation was found between the percentage of Ts cells and oligoclonal bands in CSF ($p=.001$). Ts cells showed a negative correlation with IgG-index ($p=0.01$) and oligoclonal bands in CSF ($p=0.001$). The results are in accordance with the postulated roles of T-lymphocyte subpopulations in inducing immunoglobulin changes in CSF.

12 CONCLUSION

The presentations at the Second International Workshop for Immunomodulation emphasized the close interrelationship of the nervous and immune systems. The researchers represented more than 20 countries, attesting to the widespread commitment to this relatively new area of research. The interdisciplinary approach was also evident in that immunology, neurobiology, molecular biology, clinical neurology and psychiatry, behavioral psychology, and pharmacology were represented in the research presented.

Table 1

Scientific Program for Conference on Neuroimmunomodulaton

Session 1. Neuroendocrine Correlates of Neuroimmunomodulation
Chairpersons: J.W. Hadden (US), W. Pierpaoli (Switzerland)

Invited Lectures

Brown Adipose Tissue; Its *in vivo* Immunology and Involvement in Neuroimmunomodulation, B.D. Jankovic (Yugoslavia).

Mechanisms by Which Lymphocytes Sense Their Environment, J.W. Hadden (US).

Immunoregulatory and Morphostatic Function of Bone Marrow Factors, W. Pierpaoli, G.J.M. Maestroni, E. Sache, and J. Choay (Switzerland and France).

Thymus: Key Organ Between Endocrinologic and Immunologic Systems, P.A. Deschaux (France).

Treatment of Hormone-Dependent Cancers with Analogs of Hypothalamic Hormones: Experimental and Clinical Studies, A.V. Schally (US).

Oral Presentations

The Thymus as a Neuroendocrine Organs, V. Geenen, J.J. Legros, and P. Franchimont (Belgium).

Role of the Pineal Gland in Immunity, G.J.M. Maestroni, A. Conti, and W. Pierpaoli (Switzerland).

Neuroendocrine Cells in Immune Tissues, R.H. Angeletti and W.F. Hickey (US).

Immunoregulatory Feedback Between Interleukin-1 and Glucocorticoid Hormones, A. del Rey, H. Besedovsky, E. Sorkin, and C. Dinarello (Switzerland and US)

Malfunction of the Pituitary-Thymic Axis in Aged Rats, K.W. Kelley, S. Brief, H.J. Westly, J. Novakofski, P.J. Bechtel, J. Simon, and E. Walker (US).

Identification of Pro-Opiomelanocortin mRNA in Newcastle Disease Virus-Infected Murine Splenocytes, H.J. Westly, A.J. Kleiss, K.W. Kelley, P.K. Wong, and P.H. Yuen (US).

Session 2. Enkephalins-Endorphins: Immunomodulators
Chairpersons: P. Plotnikoff (US) and J. Wybran (Belgium)

Invited Lecture

Methionine-Enkephalin: A Natural Opioid Substance with Immuno-Enhancing Properties in Man, J. Wybran and L. Schandené (Belgium).

Oral Presentations

Opiate-Induced Suppression of Antibody Production *in vivo*, R.J. Weber, A. Pert, K.C. Rice, and A.A. Hagan (US).

Enkephalins and Immunity: *in vivo* Suppression of Humoral Immune Response, B.D. Jankovic and D. Maric (Yugoslavia).

Endorphins: Involvement in Anaphylactic Shock and the Beneficial Effect of Naloxone, Naltrexone, and TRH, S. Amir (Israel).

Immunologic Effects of Met-Enkephalin, J. Chapman, T. Lee, G. Bruszer, and E. Youklis (US).

Enhancement of Host Resistance to Viral and Tumor Challenge by Treatment with Methionine-Enkephalin, R.E. Faith, A.J. Murgo, C.W. Clinkscales, and N.P. Plotnikoff (US).

Lymphocyte Production of Endorphins, E.M. Smith, D. Harbour-McMenamin, and J.E. Blalock (US).

Mechanistic Implications of the Findings that Opiates and Other Drugs of Abuse Moderate T-cell Surface Receptors and Antigenic Markers, R.M. Donahoe, C. Buesco-Ramos, J.K.A. Nicholson, J.J. Madden, and A. Falek (US).

Stimulation of Human Granulocytes by Endogenous Opioids, E.G. Fischer, and N.E. Falke (West Germany).

The Influence of Endorphins on Lymphocyte Functions, C.J. Heijnen, C. Bevers, A. Woudenberg, J. Zijlstra, and R.E. Ballieux (The Netherlands).

Bidirectional Effect of Met-Enkephalin (ME) on the Effector Functions of Phagocytic Cells, G. Foris, G.A. Medgyesi, M. Hauck, and J.T. Nagy (Hungary).

Modulation of Human Neutrophil Function by the Endogenous Opiate Enkephalins, J.J. Sich and J.D. Stinnett (US).

Session 3. Neuroendocrine Receptors in the Immune System

Chairpersons: N.R. Hall and E.M. Smith (US)

Invited Lectures

A Molecular Basis for Interactions Between the Immune and Neuroendocrine Systems, E.M. Smith (US).

Oral Presentations

Autoradiographic Visualization of Neuropeptide Receptors in Rat Spleen, C.J. Wiederman and C.B. Pert (US).

Dopamine Receptors on Isolated Membranes of Rat Thymocytes, H. Ovadia, I. Lubetzki-Korn, and O. Abramsky (Israel).

Session 4. Natural Mediators and Pharmacology of Neuroimmunomodulation

Chairperson: S.M. McCann (US)

Invited Lectures

Regulation of Lymphocyte and Mast Cell Function by Peptides of the Sensory Nervous System, D.G. Payan, J.P. McGillis, F.K. Renold, T. Chernov-Rogan, M.L. Organist, and E.J. Goetzl (US).

Hypothalamic Control of Pituitary Hormones Involved in Neuroimmunomodulation, S.M. McCann (US).

Oral Presentations

In vitro Studies of Immunoregulation by Substance P and Somatostatin, A.M. Stanisz, R. Scicchitano, D. Payan, and J. Bienenstock (Canada and US)

The Effects of Substance P on Human Eosinophil Receptors and Functions, C. de Simone, M. Ferrari, G. Ferrarelli, C. Rumi, L. Pugnaloni, and F. Sorice (Italy).

Immunomodulatory Action of Somatostatin, M. Pawlikowski, H. Stepien, J. Kunert-Radek, P. Zelazowski, and A.V. Schally (Poland and US).

Suppression of the Immune Response by Drugs Interferring with the Metabolism of Serotonin, M. Boranic, D. Pericic, M. Poljak-Blazi, V. Sverko, and T. Marotti (Yugoslovia).

Activity of Peripheral Benazodiazepines on the Oxidative Burst of P382D_1 Cells Interaction with Classical Calcium Channel Blockers, F. Zavala, M. Lenfant, and G.H. Werner (France).

Session 5. Neurophysiological Correlates of Neuroimmunomodulation

Chairpersons: O. Abramsky (Israel) and G. Renoux (France)

Invited Lectures

The Role of Nerve-Related ACHE in the Deveoplment and Function of the Thymus, K. Bullock (US).

Oral Presentations

A Neurophysiological Correlate of an Immune Response, D. Saphier, O. Abramsky, S. Feldman, G. Mor, and H. Ovadia (Israel).

Influences of Bilateral Neocortex Ablation on the Immune System, G. Renoux, K. Bizière, M. Renoux, D. De Genne, and J.M. Guillaumin (France).

Effect of Hypothalamic Lesions on Experimental Autoimmune Disease in Rats, O. Abramsky, T. Brenner, E. Wertman, R. Mizrachi, and H. Ovadia (Israel).
Hypothalamic Lesions Decrease Tracheal Anaphylaxis, A.J.M. van Oosterhout and F.P. Nijkamp (The Netherlands).
Effects of Hypothalamic Lesions in Mice Upon Lymphocyte Subsets, M. Katayama, S. Kobayashi, N. Kuramoto, and M.M. Yokoyama (Japan).
Effect of Sympathetic and Parasympathetic Denervation Upon the Immune Response, M. Braun, A. Auto, R. Baler, M. Carlomagno, H. Romeo, and D. Cardinalli (Argentina).
Effects of Brain Injury on Systemic Antibody Responses and Low Zone Tolerance, T. Quirico-Santos and H. Valdimarsson (Brazil and Iceland).
Role of the Sympathetic Nervous System in the Formation of Circadian Rhythms in Immune Response, B. Radosevic-Stasic, L. Polic and D. Rukavina (Yugoslavia).
The Involvement of Brain Monoaminergic Structures in the Effect of Immunomodulators, O. Kadlecova, K. Masek, and P. Petrovicky (Czechoslovakia).
Muramyl Peptides as Mammalian Physiological Modulators, J.W. Karaszewski and J.M Krueger (US).

Session 6. Stress and Immunity
Chairpersons: B.H. Fox and L. Temoshok (US)

Invited Lecture
Type A Behavior and Cancer Mortality: Preliminary Data and Planned Follow-Up, B.H. Fox, D.R. Ragland, R.J. Brand, and R.H. Rosenman (US).

Oral Presentations
Psychological Stress Damages DNA, H.K. Fischman, D. Kelly, and R. Pero (US).
The Influence of Various Forms of Emotional Stress on the Immune System, R.E. Ballieux, G. Croiset, H.D. Veldhius, D. deWied, F. Berkenbosch, B. Bohus, and C.J. Heijnen (The Netherlands).
Pharmacological Interventions to Antagonize Stress Immune Consequences, H. Raskova, L. Celeda, Z. Urbanova, J. Vanacek, and A. Kubicek (Czechoslovakia).
The Impact of Mild Emotional Stress Induced by the Passive Avoidance Procedure on Immune Reactivity, G. Croiset, H.D. Veldhuis, C. Bevers, R.E. Ballieux, D. deWied, and C.J. Heijnen (The Netherlands).
Effect of Diazepam on Brain Neurotransmitters, Plasma Cortiosterones, and Immune Systems of Stressed Rats, D. Pericic, H. Manev, M. Boranic, M. Poljak-Blazi, and N. Lakic (Yugoslavia).
Changes in the Population of T-cell Subsets in Stressed Mice, H. Teshima, H. Sogawa, H. Kihara, S. Nagata, and Y. Ago (Japan).
Conditioned Immunodepression: A New Methodological Approach, P.J. Neveu, F. Crestani, and M. LeMoal (France).
Stress-Induced Modulation of the Immune Network, R. Bosing-Schneider (West Germany).

Session 7. Sleep and Immune Functions
Chairperson: J.M. Krueger (US)

Oral Presentations
Immune Modulators as Sleep Promoters, J.M. Krueger (US).
Muramyl Peptides, Serotonergic System, and Sleep, K. Masek (Czechoslovakia).

Session 8. Behavior: Associative Learning and Immunity
Chairpersons: R. Ader and N.H. Spector (US)

Invited Lectures
Behavioral Modulation of Immunity, R. Ader (US).
Old and New Strategies in the Conditioning of Immune Responses, N.H. Spector (US).

Oral Presentations

Conditioned Enhancement of Delayed Type Hypersensitivity, N. Cohen, D. Bovbjerg, and R. Ader (US).

Regulation of Natural Immunity (NK Activity) by Conditioning, R.N. Hiramato and V.K. Ghanta (US).

Conditioned Taste Aversion in Rats Subjected to Anaphylactic Shock, V.J. Djuric, B.M. Markovic, M. Lazarevic, and B.D. Jankovic (Yugoslavia).

Classically Conditioned Cyclophosphamide Effects on White Blood Cell Counts in Rats, S. Klosterhafen and W. Klosterhafen (West Germany).

Prior Stress and Behaviorally Conditioned Histamine Response, K.A. Dark, H.V.S. Peeke, G. Ellman, C. McCurry, and M. Salfi (US).

Suppression of Plaque-Forming-Cell Responses by Classical Conditioning with Cyclophosphamide, D. Bovbjerg, Y.T. Kim, G.W. Siskind, and M.E. Weksler (US)

Psychopharmacological Activity of Immune Complex in Rat is Complement Dependent, N. Schupf and C.A. Williams (US).

Session 9. Cancers, AIDS, and Immunity

Chairpersons: G.F. Solomon and R.N. Hiramoto (US)

Invited Lectures

Enkephalins and Endorphins, Behavioral Stress and Immunomodulators in Normal Volunteers and Cancer Patients (AIDS), N.P. Plotnikoff, G.C. Miller, N. Nimeh, R.E.F. Aita, A.J. Murgo, and J. Wybran (US and Belgium).

Psychoneuroimmunologic Considerations in AIDS, G.F. Solomon (US).

Oral Presentations

An Intensive Psychoimmunologic Study of Long-Surviving Persons with AIDS, G.F. Solomon, L. Temoshok, D.P. Stites, and J. Zich (US).

Neuropeptides Modulating Macrophage Function, R. Peck (Switzerland).

Stress-Behavior Interaction in Hamster Tumor Growth, L. Temoshok, H.V.S. Peeke, C.W. Mahard, K. Axelsson, and D.M. Sweet (US).

Influence of Conditioned Natural Immunity on Tumor Growth, V. Ghanta, R.N. Hiramoto, N.A. Spector (US).

Session 10. Psychiatric and Neurological Disorders with Immunological Features

Chairpersons: C.A. Williams (US) and G.I. Kolyaskina (USSR)

Invited Lectures

The Neuropharmacology of Immune Complex Activity in the Rat Hypothalamus, C.A. Williams (US).

A Psychiatrist's View of Neuroimmunomodulation: The Neuro-Immune Interactions and Mechanisms, M.E. Vartanian and G.I. Kolyaskina (USSR).

Oral Presentations

Clinical and Laboratory evidence of Autoimmune Disease in Acute Schizophrenia, R. Ganguli, B. Rabin, R. Alexander, M. Lyte, U. Ragu, and R. Kelly (US).

Anti-Brain Antibody in Patients with Schizophrenia, B. Rabin, R. Ganguli, R. Kelly, R. Alexander, and M. Lyte (US).

Production of Interleukin-2 in Schizophrenia, F. Villemain, L. Chatenoud, E. Guillibert, Y. Pelicier, and J.F. Bach (France).

Immune Function and Immune Genetics in Psychiatric Patients, N. Müller, M. Ackenheil, R. Eckstein, E. Hofschuster, and W. Mempel (West Germany).

Hypnosis and Delayed Hypersensitivity, S.E. Locke, B.J. Ransil, N.A. Covino, J. Toczdlowski, C.M. Lohse, H.F. Dvorak, K.A. Arndt, and F.H. Frankel (US).

Imagery and Relaxation in Neuroimmune Function, M.L. Jasnoski and J. Kugler (US and West Germany).

The Influence of Emotional State on the Mobilization of Marginal Pool Leucocytes Following Insulin Induced Hypoglycemia, R.N. Melmed, D. Roth, and E. Edelstein (Israel).

The Correlation of T-lymphocyte Subpopulaton Levels to IgG-index and to Oligoclonal Immunoglobulin Bands in the CSF, T. Pirttila, J. Antonen, R. Oikarinen, and H. Frey (Finland).

Immunological and Clinical Evaluation of Multiple Sclerosis Patients Treated with Steroids and/or Calf Thymic Hormones, M.P. Dabrowski, B.K. Dabrowska-Bernstein, A. Stasnik, K. Gajkowski, and S. Korniluk (Poland).

Interleukin-2 Receptor Expression on Peripheral Blood Leukocytes in Multiple Sclerosis Patients, K. Selmaj, C. Plater-Zyberk, K.A. Rockett, R.N. Marni, R. Alaim, G.D. Perkin, and F.C. Rose (Poland and UK).

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